Rapid and cost-effective fabrication of selectively permeable calcium-alginate microfluidic device using "modified" embedded template method

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In this paper, we have presented a non-lithographic embedded template method for rapid and cost-effective fabrication of a selectively permeable calcium-alginate (Ca-alginate) based microfluidic device with long serpentine delay channel. To demonstrate the versatility of the presented method, we have demonstrated two different strategies to fabricate serpentine long delay channels without using any sophisticated microfabrication techniques, in formal lab atmosphere. The procedure presented here, also, enables the preparation of a multilayered microfluidic device with channels of varying dimensions, in a single device without using any sophisticated micromachining instrumentation. In addition, we have also qualitatively studied the diffusion of small and large molecules from a Ca-alginate based microfluidic device and proposed a method to effectively control the out-flow of macro biomolecules from the crosslinked Ca-alginate matrix to create a selectively permeable matrix required for various biological and biomimetic applications, as mentioned in the Introduction section of this work. © *2012 American Institute of Physics*. [doi:10.1063/1.3672189]

INTRODUCTION

Biomaterial based microfluidic devices have gained enormous attention, recently. Due to their biological origin, biomaterials make a better interface between biomolecules and biomaterial based microstructured scaffolds for applications such as tissue engineering, cell culture, prosthetic implantation, cell free protein synthesis, etc.[1] However, the fabrication of microstructures, especially microfluidic devices using biopolymers, is a challenging area because of the softness of the biopolymers. In addition, controlling diffusion of molecules in/out of biomaterial based microfluidic devices has not yet been significantly addressed.[2] Moreover, tuning or altering the material properties of natural or synthetic biopolymers after or before fabrication is still a challenge. Cabodi *et al.* suggested criteria for a successful microfluidic biomaterial and demonstrated fabrication of calcium-alginate (Ca-alginate) microfluidic device, which fulfills all the critical characteristics required for a successful biomaterial for microfluidic applications.[3] However, success rate of the fabrication of Ca-alginate microfluidic device, as suggested by Stroock's group,[1–3] depends on skills of an individual.[4] Furthermore, sealing of patterned Ca-alginate with a flat piece of alginate is also hard to control. Moreover, controlling mass transfer of molecules in alginate microfluidic device including selective permeability of alginate matrix for micro and macro molecules

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remains a challenge to broaden the applications of Ca-alginate devices in various other fields, such as microchemotaxi,[5] generation of molecular gradients,[6] biocatalytic applications such as encapsulation of enzymes in enzyme-based field effect transistors (ENFETs),[7] cell free protein expression,[8] membrane of hollow fiber field flow fractions (FFFs),[9] enzyme encapsulated selectively permeable membranes for biosensors,[10] artificial kidney on-chip,[11] artificial functional lung system[12] or even artificial vascular system,[13] tissue engineering,[2] and many others. We have previously demonstrated a facile embedded template method for the fabrication of various polymers with varying dimensions and shapes, including Ca-alginate device.[14] However, making microfluidic device with serpentine long delay channels without using any sophisticated microfabrication techniques still remains a challenge, especially when the soft biomaterial like alginate is used for the fabrication of microfluidic device. In the current work, we have presented, for first time, two different strategies to fabricate serpentine long delay channels, in non-cleanroom atmosphere, using non-lithographic embedded template method for rapid and cost-effective fabrication of a Ca-alginate based microfluidic device. The procedure presented here also enables the fabrication of a multilayered microfluidic device with channels of varying dimensions, in a single device without using any sophisticated micromachining instrumentation. In addition, we have also qualitatively studied the control of diffusion based mass transfer of micro- and macro-molecules from Ca-alginate based microfluidic device. Also herein, we propose a method to effectively control the mass transfer of macro biomolecules from the crosslinked alginate matrix, after coating with different concentrations of chitosan, which leads to selective permeability of Ca-alginate matrix, desired for various applications.

EXPERIMENTAL

In the current report, we have made an attempt to report two different approaches to fabricate Ca-alginate microfluidic devices with long serpentine channels using the embedded template method as described in our previous work.[14] All tubings and capillaries used in this work were purchased from Upchurch Scientific (Oak Harbor, WA) unless otherwise mentioned in the text. High gel-strength, low-viscosity sodium alginate (Protanal LF 10/60 FT) was obtained as a kind gift from FMC Biopolymer, Norway for the fabrication of Ca-alginate microfluidic channels. During the first approach, to fabricate an alginate microfluidic device, template framework was assembled using tubings/capillaries of different sizes, as depicted in Fig. 1(a). Initially, two sets of fused Si capillaries (O.D. 360 μm, I.D. 100 μm), connected at both the ends with small pieces of fluorinated ethylene propylene (FEP) tubing (O.D. 1.5 mm and I.D. 508 μm), were placed $\sim$32 mm apart from each other, on a double sided tape (depicted as vertical pair in Figure 1(a)). Subsequently, a second layer of framework containing 4 sets of fused Si capillaries with only one end connected to FEP tubing (Fig. 1(a)) was placed perpendicular to the 1st layer of frame work, maintaining a distance of 6 mm from each other.

In the second approach, as shown in Figs. 2(a)–2(c), we have used "modified" embedded template method to prepare a microfluidic device, in more reproducible manner, with smaller channel dimensions. In this method, primarily, a polydimethylsiloxane (PDMS) support frame/boat was made by molding Sylgard 184 (Dow Corning, MI) prepolymer against a stainless steel block glued with 4 small pieces of FEP tubing (O.D. 1.5 mm and I.D. 508 μm) on both the ends, as shown in Fig. 2(a). The PDMS boat was then plasma bonded to glass coated with 0.1% polyethylenimine (PEI, M_w 100 000, Sigma-Aldrich, MA) solution as depicted in Fig. 2(b). An embedded template framework, to fabricate a Ca-alginate microfluidic device, was assembled using commercially available FEP tubing of O.D. 250 μm and I.D. 200 μm (Zenus, Orangeburg, SC) and FEP tubing (O.D. 1.5 mm and I.D. 508 μm), as shown in Fig. 2(c).

To fabricate Ca-alginate microfluidic devices, either the scaffolds or framework were placed in the petri dish, followed by submerging the entire frame work in a 4% solution of sodium alginate in distilled water (Figs. 1(b)) and 2(d)). Please note that in case of second framework only the void space inside the PDMS walls was filled with 4% solution of sodium alginate. The alginate solution was then covered with a 0.2 mm track-etched polycarbonate membrane (Whatman, Kent, UK) and, subsequently, with a perforated glass plate. The Ca-alginate gel microfluidic

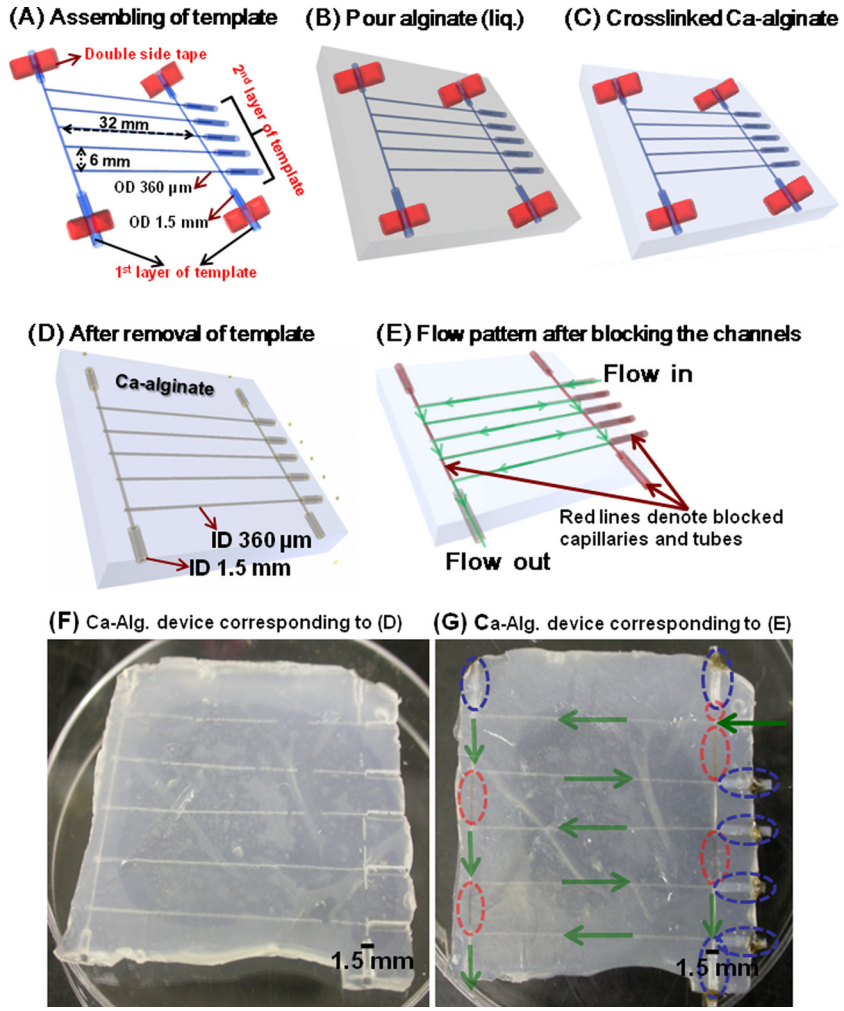

FIG. 1. Schematic of the fabrication of long delay channel Ca-alginate microfluidic device using embedded template method (Scheme not to the scale). Please see text for full description.

structure was formed by placing the entire petri dish in 60 mM calcium chloride solution for 3 h (Figs. 1(c) and 2(d)). After 3 h, template tubings were carefully pulled out from the solidified Ca-alginate device to create the microfluidic channels (see Figs. 1(d) and 2(e)).

To connect all the four channels of the device, as shown in Fig. 2(e), a small piece of FEP tube (O.D. 1.5 mm and I.D. 250 μm) was used as revealed in Fig. 2(f). A multilayer device with top channel dimensions of 360 μm and below layer of 250 μm was also fabricated using the "modified" embedded template method as shown in Figs. 3(a) and 3(b). For the chitosan (Sigma-Aldrich, MA) coating, single channel devices were fabricated using fused silica tubing of O.D. 360 μm.

RESULT AND DISCUSSION

Fabrication

In this study, instead of the mold fabricated by sophisticated lithography techniques, a template frame work composed of tubing with different diameters and sizes were used to prepare microfluidic devices, which can be achieved in any conventional laboratory conditions without any special facilities. This work is an extension of our previously published work,[14–16] where we have demonstrated the fabrication of a very simple device; however, making a long

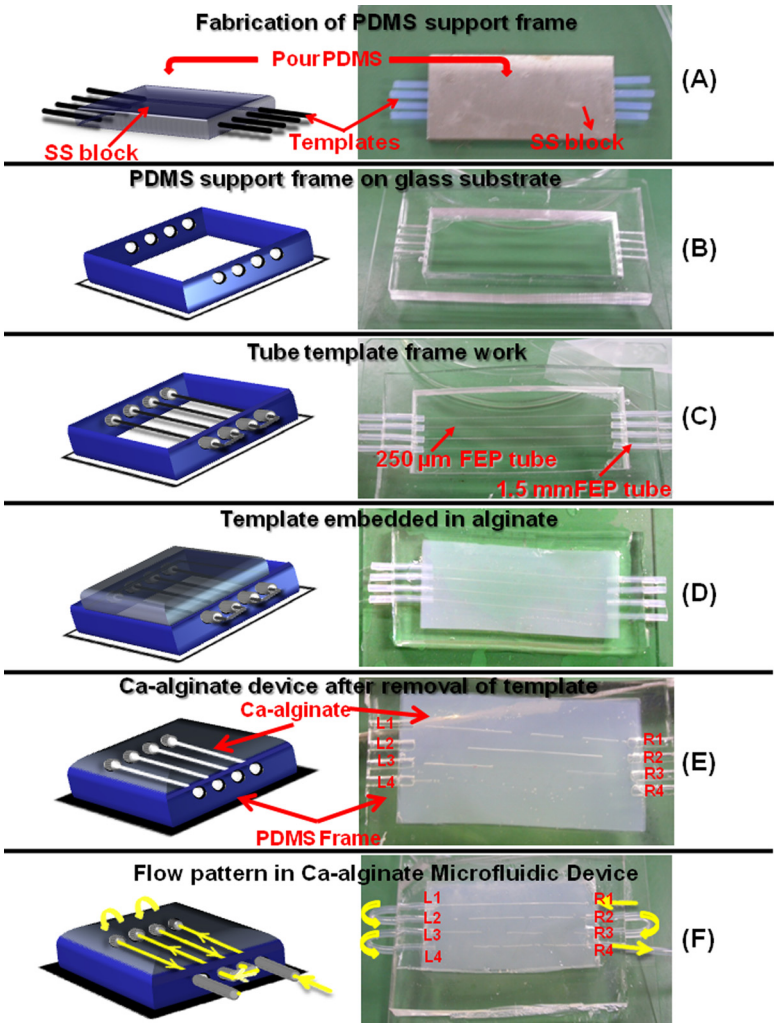

FIG. 2. Schematic of modified embedded template method for the fabrication of serpentine long delay channel Ca-alginate microfluidic device (Scheme not to the scale). Please see text for full description.

serpentine delay channel was still a challenge. Consequently, in the present work, we have proposed two approaches to make long delay channels using embedded template method.

Figures 1 and 2 show a schematic and corresponding optical pictures of the non-lithographic embedded template fabrication process. The framework created to make the microfluidic channel was submerged in Na-alginate solution and crosslinked with $CaCl_2$ solution for 3 h. After solidification of alginate matrix, the capillaries and tubings used as embedded templates were carefully pulled out leaving a void (micro-channel) space in place of the capillaries (Figs. 1(d) and 1(f) and 2(e)). Because all the tubing and capillaries, used in this work, possessed low surface energy, it was very easy to pull the templates out from the crosslinked Ca-alginate matrix without causing any damage to the matrix. The void space created a circular fluidic channel, which is essential for many biological applications. However, the channels prepared by following method 1 (Fig. 1) were all interconnected. In order to create a near-serpentine fluidic pathway (Figs. 1(e) and 1(h)), we inserted block capillaries of 6 mm length and 360 μm O.D., in the areas shown as red lines in Fig. 1(e) inside the ca-alginate microfluidic device as shown in Fig. 1(g); while the 1.5 mm I.D. channels of device were blocked by FEP tubing (O.D. 1.5 mm). The inserted block capillaries and tubes are shown in Fig. 1(g) under the red doted circles and blue circles, respectively. The flow pattern inside the Ca-alginate microfluidic device, after the insertion of blocked tubings and capillaries, is shown as green lines as depicted in Figs. 1(e) and 1(g).

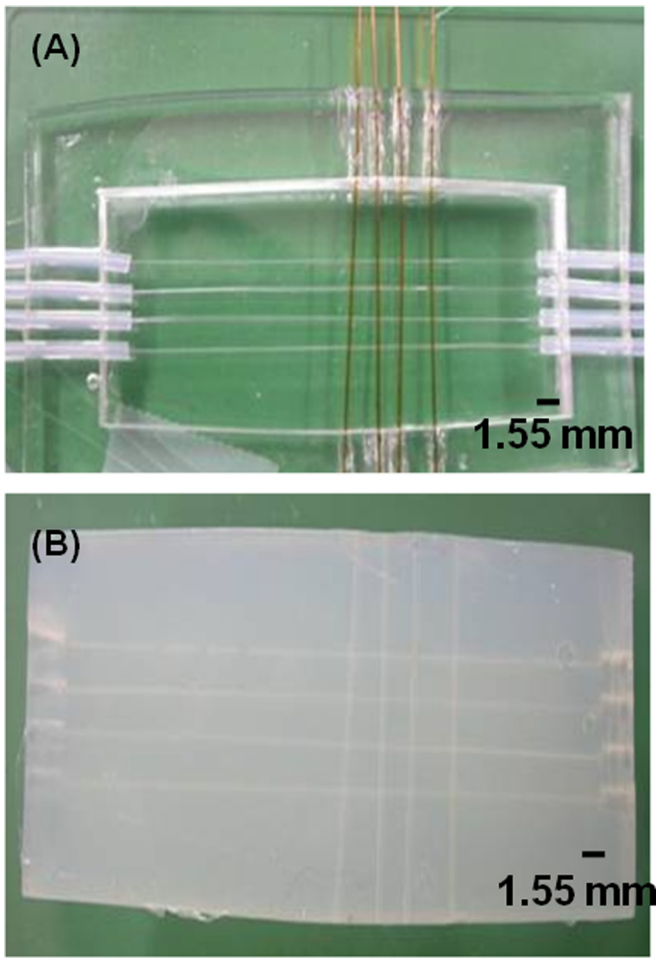

FIG. 3. Fabrication of multilayered Ca-alginate device using modified embedded template method. (a) Framework of double layered embedded template. (b) Ca-alginate microfluidic device after removal of templates.

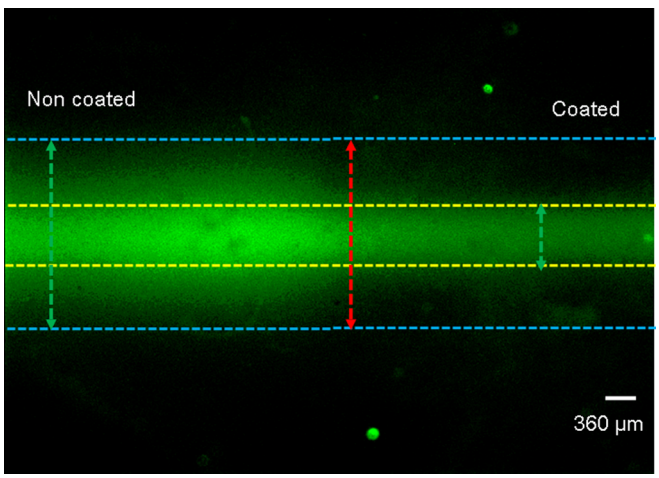

FIG. 4. Fluorescence microscopic image of Ca-alginate device half coated with chitosan, showing diffusion of GFP in non-coated part of the channel.

In order to prepare smaller dimension channels in a much more reproducible manner, we proposed a "modified" version of embedded template method, where in place of double sided tape, a PDMS support frame was used to make embedded template framework (Figs. 2(a) and 2(b)). Schematic of modified embedded template method alongside the corresponding device pictures is shown in Figs. 2(a)–2(f). The FEP tubes attached to the stainless steel block (Figs. 2(b) and 2(c)) eventually created "adopter ports" to connect the alginate microchannels. The connections were made using sets of FEP tubing (1.5 mm O.D., 250 μm I.D.) as shown in Fig. 2(f) The holes created in the PDMS boat (Fig. 2(b)) were used to fit-the FEP tubings. Through these FEP tubings, another FEP (O.D. 250 μm, I.D. 200) was carefully sewed through to create four straight

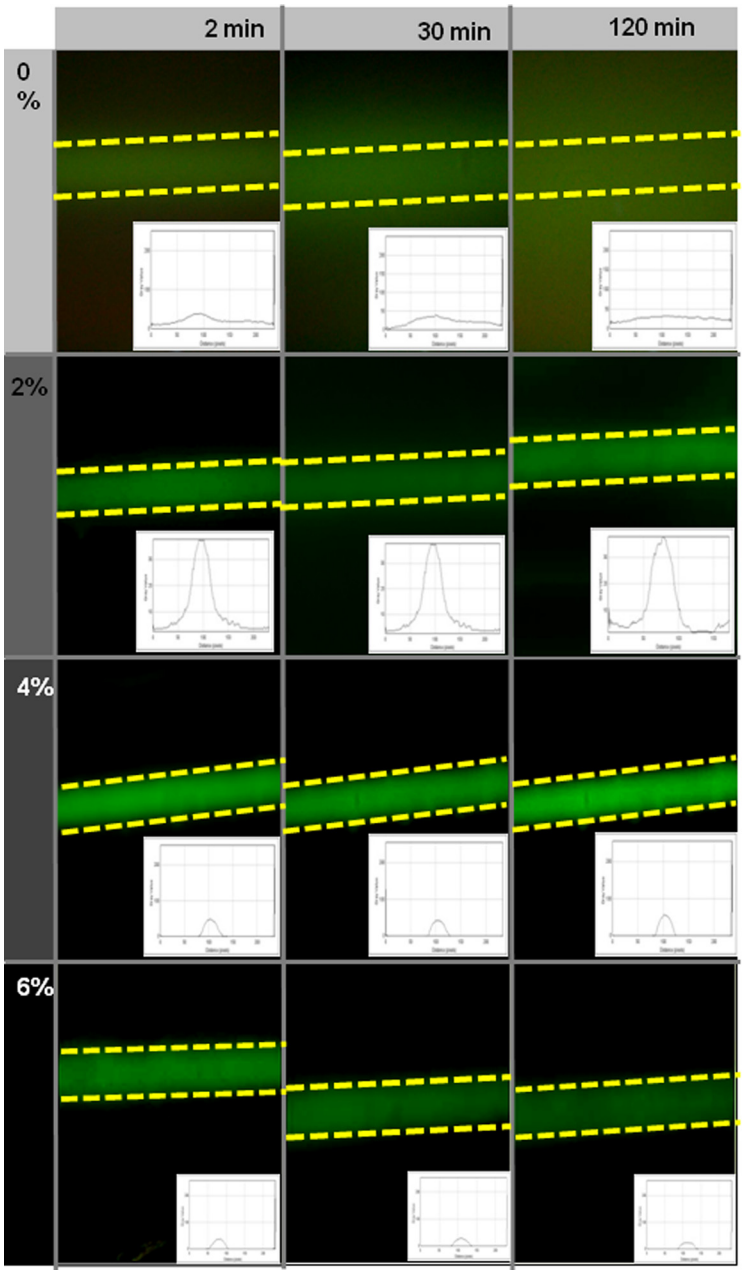

FIG. 5. Control of diffusion mass transfer of GFP from microchannel into Ca-alginate matrix at different periods both before and after coating with different concentrations of chitosan. Inset represents the image analysis of gray scale vs. distance in pixel.

channel templates. After cross-linking of alginate with $CaCl_2$ for 3 h, thinner FEP tubing was cut from the end and carefully pulled out to create 250 μm circular fluidic channels, corresponding to O.D. of 250 μm of FEP tubing. To create a long delay channel, outlet port adapter L1 (Figs. 2(e) and 2(f)) of first channel was connected to L2 port using a piece of FEP tube (O.D. 1.5 mm and I.D. of 250 μm). Similarly, R2 port was connected to R3, and L3 was connected to L4 to create a continuous long delay channel of 250 μm I.D. (Fig. 2(f)). Ports R1 and R4 worked as inlet and outlet ports, respectively.

To show the further advantages of this facile fabrication method, we employed modified embedded template method to create a multilayer device with different dimensions as shown in Figs. 3(a) and 3(b). The top layer consisted of 360 μm I.D. of channel, while the bottom layer corresponded to a 250 μm I.D. channel.

Controlling permeability of metal alginate gel

Diffusion of both micro and macro-molecules is essential for any biomaterial based microfluidic device used as a 3D scaffold. The diffusion can also be termed as a permeability of small and large molecules.[5] It is well known that cross-linked metal-alginate gel consists of wide pore size distribution due to the open lattice structure of the matrix.[17,18] It has been reported that low molecular-weight molecules, such as glucose and α-lactalbumin, can diffuse in and out of matrix

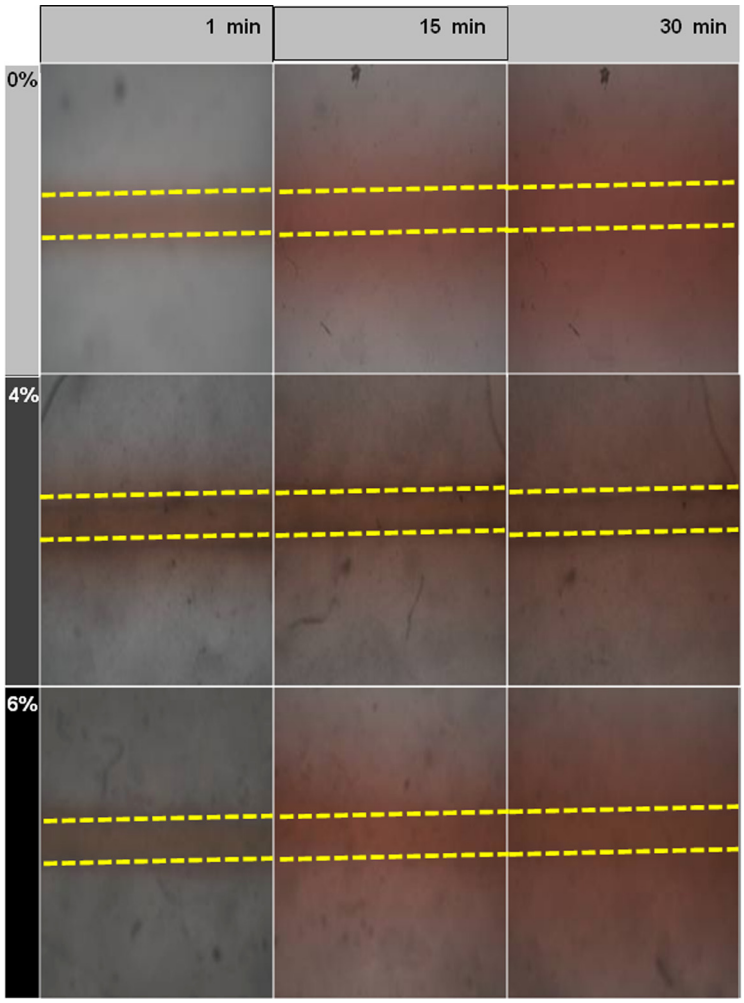

FIG. 6. Diffusion of phenol red into the alginate matrix through the microchannel both before and after coating with chitosan at different time intervals.

without any restraint.[18] While higher-molecular-weight species, such as albumin and gamma-globulin can diffuse out of the matrix but cannot diffuse into the matrix.[19] A quantitative diffusion study carried out by Choi *et al*. suggests that the pore size of Ca-alginate is about 3.6 nm, larger than the radius of gyration of Fluorescein isothiocyanate labelled bovine serum albumin (FITC-BSA) molecule (MW 60 kDa).[2] Different types of coatings such as with chitosan,[20–22] poly-(lysine)/alginate,[22,23] and other materials have been used as a barrier to stop or reduce the diffusion of encapsulated macromolecules. To check the above-mentioned statements and to evaluate the effect of coating of alginate, we conducted series of experiments by coating the inner walls of alginate microfluidic device with different concentrations of chitosan. Green fluorescence protein (GFP, MW 27 kDa) and phenol red (MW 354.38 g mol^{-1}) were used as a model molecule to observe diffusion mass transfer through the alginate matrix. As an initial experiment, an alginate microfluidic channel, created by a 360 μm capillary template, was half coated with 2% solution of chitosan. The half coating of inner wall was performed by blocking the rest half of the channel with a fused silica capillary. After removing the excess of chitosan and successive washing, the single channel alginate microfluidic channel (Fig. 4) was first filled with GFP. It was observed that GFP molecules, although a macro molecule, can diffuse into the matrix without any delay. However, on the coated side of the channel, negligible diffusion of alginate was observed (Fig. 4). A series of experiments with varying concentration of chitosan starting from 0% to 6% chitosan solutions were performed to evaluate the effect of chitosan concentration on inward diffusion of macro molecules, such as GFP (Fig. 5). It is evident from the results illustrated in Fig. 5, that even chitosan concentration as small as 2% is sufficient to avoid diffusion of macromolecule into the matrix. This indicates that chitosan coating does not allow GFP molecules to diffuse into the matrix. Based on the radius of gyration of GFP (MW 27 kDa) $\sim$ 1.74 nm,[24] after coating Ca-alginate with even 2% chitosan solution, pore size of Ca-alginate matrix reduced to $<$1.74 nm. To further evaluate the effect of coating on diffusion mass transfer of small organic molecules such as phenol red (MW 354.38 g mol^{-1}), experiments similar to that mentioned for GFP diffusion were performed. It was observed that phenol red readily diffused into the bare alginate matrix as well as into the chitosan-coated matrix without any delay (Fig. 6), even at higher chitosan concentration of 6%. From these experiments, it was concluded that alginate matrix coated with chitosan could be used to make alginate matrix with selective permissibility for small organic molecules. This kind of selectively permeable alginate microfluidic device holds tremendous potential in biological applications, as indicated in the Introduction section.

CONCLUSION AND FUTURE DIRECTION

In this article, we have successfully demonstrated two simple, cost effective single-step, rapid methods for the production of microfluidic device with long serpentine delay channels. The demonstrated "modified" embedded template methods can be used in normal laboratory atmosphere without much of hassles and without any prerequisite of sophisticated microfabrication facility. Furthermore, this method is particularly attractive for third world countries to initiate simple microfluidic related work, where they lack sophisticated fabrication facilities. In addition, we have demonstrated that coating of chitosan can reduce the pore size of Ca-alginate matrix from reported 3.3 nm to 1.74 nm. This reduced pore size restricted the diffusion of macro biomolecules into the Ca-alginate matrix, while permitted free diffusion of micro-molecules. Such kind of selectivity of alginate can be easily applied in 3D cell culture scaffold with embedded microfluidic channels for temporal and spatial control of diffusion mass transfer of micromolecules, such as growth factors, nutrients, and metabolites, while not allowing mass transfer of extra cellular molecules. Such kind of functional microfluidic device can be used to design bioreactors,[25] artificial organs,[26–28] cell free protein synthesis system, microchemotaxis, and biocatalytic microbioreactors.

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